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Analysis of Leaf Pigments in Cultivars and Samples of *Phaseolus aureus* L.

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Abstract

In this study, a quantitative analysis of leaf pigments was performed in various cultivars and accessions of *Phaseolus aureus* L. originating from different geographical regions. During the research, the contents of chlorophyll "a", chlorophyll "b", and carotenoids were determined spectrophotometrically using samples collected from the true leaves. The results showed that chlorophyll "a" had the highest concentration among the investigated pigment types, confirming its dominant role in the photosynthetic process. Chlorophyll "b" levels varied moderately among cultivars; however, no direct evidence was obtained regarding its association with shade-adapted or medium-light growing conditions. Therefore, the observed differences in chlorophyll "b" content are interpreted as cultivar-specific physiological characteristics rather than light-adaptation traits. Carotenoids demonstrated a notable protective function against photo-oxidative stress, with the highest concentrations detected in accessions originating from Afghanistan, China's Mainland, and Uzbekistan. The findings provide an important scientific basis for evaluating ecological adaptability, photosynthetic activity, and potential productivity of *Phaseolus aureus* cultivars.

Keywords

Phaseolus aureus L., Leaf Pigments, Chlorophyll "a", Chlorophyll "b", Carotenoid, Spectrophotometry, Photosynthesis, NC-17376 China, NC-17338 India, NC-33641 Afghanistan, NC-33068 Uzbekistan, NC-32000 Vietnam, NC-32061 Philippines, NC-17385 Manchuria, NC-17397 Vietnam, NC-33647 Taiwan Region, NC-17393 China-2019, Turon, Durdona

1. Introduction

In recent years, ensuring global food security has become one of the most pressing challenges facing humanity. According to the Food and Agriculture Organization

of the United Nations (FAO), the growing world population has significantly increased the annual demand for food. At the same time, climate change, water scarcity, land degradation, and various environmental issues are exerting considerable pressure on the stability of agricultural production [1].

The Asian region, particularly the Central Asian countries—including Uzbekistan—experiences arid climatic conditions and limited water resources, which necessitate the introduction of efficient, short-season, and nitrogen-fixing crop species into agricultural systems. In this context, *Ph. aureus* is of strategic significance as a food, forage, and soil-improving leguminous crop.

This crop's agronomic advantages—rapid maturation (60 - 90 days), high tolerance to heat and drought, and ability to fix atmospheric nitrogen—make it suitable for cultivation as a secondary (after-harvest) crop under the conditions of Uzbekistan [2].

In recent years, the President of the Republic of Uzbekistan has adopted several strategic documents, including the "Food Security Strategy of the Republic of Uzbekistan for 2020-2030" [3], the "Agricultural Development Strategy of the Republic of Uzbekistan until 2030" (Decree of the President № 4576, January 23, 2019) [4], and the Resolution "On Measures for the Development of Scientific Research in Agriculture" (Decree of the President № 3857, July 17, 2018) [5]. These documents emphasize the introduction of resource-efficient, high-yielding, and environmentally sustainable crop species as a priority direction.

In addition, the Law of the Republic of Uzbekistan "On Food Security" (June 20, 2023, Law of the Republic of Uzbekistan № 868) [6] legally guarantees the population's access to nutritious, safe, and high-quality food products at the national level.

Therefore, studying the cultivation technology of *Ph. aureus* under the conditions of Uzbekistan and implementing resource-saving agricultural practices constitutes an important scientific direction contributing to food security and sustainable agricultural development in the country [7].

2. Object and Methods of Research

The research was conducted on 12 cultivars of *Ph. aureus*: NC-17376 China, NC-17338 India, NC-33641 Afghanistan, NC-33068 Uzbekistan, NC-32000 Vietnam, NC-32061 Philippines, NC-17385 Manchuria, NC-17397 Vietnam, NC-33647 Taiwan region, NC-17393 China-2019, Turon and Durdona.

These cultivars were officially registered and recommended for cultivation by the "Center for Variety Testing of Agricultural Crops".

For laboratory analysis, samples were collected from the cultivars at the true leaf stage. There are several scientific reasons why the true leaves of the plant were selected for pigment analysis:

Fully developed photosynthetic apparatus. True leaves are morphologically and physiologically mature, containing fully developed chloroplasts. Therefore, their pigment composition accurately reflects the actual photosynthetic activity of the

plant.

Stable pigment concentration. Pigment content in cotyledons or young leaves changes rapidly due to ongoing developmental processes. In contrast, true leaves represent a more stable stage of pigment synthesis and degradation, making them a reliable material for spectrophotometric analysis.

Lower sensitivity to external stress factors. Very young or senescing leaves tend to exhibit strong pigment fluctuations in response to environmental stress. True leaves, being in an optimal physiological phase, provide data that more accurately represent the plant's natural physiological state.

Clear differentiation of cultivar-specific traits. Genetic differences in pigment content among cultivars and accessions are most consistently expressed in true leaves. For this reason, true leaves offer a scientifically sound basis for comparing pigment profiles across varieties or populations.

Compliance with methodological standards. Standard methodologies for plant pigment analysis (e.g., Arnon, Lichtenthaler, and Wellburn methods) recommend using mature, fully developed leaves for the preparation of pigment extracts.

In conducting the research, cultivar selection and field sowing followed the methodology of Dospeskhov [8]; agro-technical activities were based on the methods of Mansurov *et al.* [9]; field experiments were carried out according to Dospeskhov [8]; chlorophyll content in leaf tissues was determined following the classical methodology of Arnon (1949) [10]; and statistical analysis was performed using the methods of Fisher [11]. The obtained data were systematized in tabular form using Microsoft Excel.

The study was conducted at the Research Center and Experimental Field of the Department of Medicinal Plants and Botany of Gulistan State University, Syrdarya Region.

Syrdarya Region is an administrative region of the Republic of Uzbekistan, established on February 16, 1963. It borders Kazakhstan to the north, Tashkent Region to the east, Tajikistan to the south, and Jizzakh Region to the west.

The region's relief mainly consists of undulating plains, descending from the southeast toward the northwest. A portion of the Mirzachul steppe lies within its territory. Elevation reaches 230 m in the north, 400 - 450 m in the central part, and 600 - 650 m in the south and southwest. The wide Syrdarya Valley occupies its eastern part.

The climate is sharply continental and arid, with an annual average temperature of 14˚C. Hot desert winds often dry the soil and negatively affect plant development. The vegetation period lasts 218 days. Annual precipitation ranges from 180 - 220 mm, falling mainly in winter.

Soils are predominantly light-colored, weakly developed grey soils, slightly to moderately saline, with loamy and clay-loamy texture. Salt-affected soils also occur in some areas.

The regional flora includes a mixture of steppe, desert, and cultivated species. Among cultivated and wild plants, economically important species include *Tulipa*

gesneriana (tulip), *Carthamus tinctorius* (safflower), *Capsicum annuum* (pepper), *Mentha* spp. (mint), *Spinacia oleracea* (spinach), *Amygdalus* spp. (almond), *Hordeum vulgare* (barley), and *Glycyrrhiza glabra* (licorice). Native steppe and desert species include *Salsola* spp., *Artemisia* spp., *Haloxylon* spp., *Calligonum* spp., and various *Acacia* and *Tamarix* species. [12].

3. Results and Analysis

Ph. aureus is an annual plant belonging to the family *Fabaceae* and holds significant importance in food production and agriculture. Synonyms of *Ph. aureus* include *Phaseolus radiatus* and *Vigna radiata* [13]-[16]. *Ph. aureus* is believed to have originated in South Asia, particularly on the Indian subcontinent [17]. At present, it is widely cultivated in tropical and subtropical regions, including Asia, Africa, Australia, and South America [18].

Ph. aureus is divided into subspecies distributed across India, China, and Iran. The mung bean originates from Southwestern Asia and has been cultivated since the 4th - 3rd millennia BCE. Today, this crop is widely grown in Central Asia, India, Pakistan, Afghanistan, Iran, China, Japan, and other countries. The plant has a taproot system reaching depths of up to 1.5 meters. The well-developed roots engage in symbiotic nitrogen fixation with *Rhizobium* bacteria [19], a feature essential for enhancing soil fertility. Its stem is erect or slightly spreading, reaching a height of 30 - 120 cm. The stem is slender and sometimes pubescent [20]. The leaves are broad and large; the flowers are bisexual, papilionaceous, and arranged in groups of 3 - 12 in the axils. The leaves are compound with three leaflets, green, and oval-shaped, with a length of 5 - 10 cm [21].

The flowers are yellow or yellow-green. They are small (1 - 2 cm), yellow-colored, and possess the typical structure of the Fabaceae family. Although primarily self-pollinating, cross-pollination by insects is also possible [17]. The fruits are cylindrical pods, 5 - 10 cm long, containing 10 - 20 seeds. When ripe, the pods turn brown or black [20]. The seeds are small, usually green, and sometimes yellow or black. Seed diameter is 2 - 5 mm, with a mass of 30 - 60 mg; the 1000-seed weight ranges from 40 - 80 g [18]. The plant's vegetation period lasts 60 - 90 days, varying by cultivar and environmental conditions [22]. Predominantly self-pollinating, the species maintains genetic stability, although occasional insect-mediated pollination (e.g., by bees) may occur [21].

To determine the chlorophyll and carotenoid pigments in the leaves of selected cultivars, analyses were conducted based on D. I. Arnon's method "Spectrophotometric Determination of Chlorophylls in Leaf Tissue". This method is designed to quantify the major pigments of photosynthesis—chlorophyll "a", chlorophyll "b", and total chlorophyll content—in plant leaves. The research was carried out using the spectrophotometric approach proposed by Arnon (1949) [10]. The essence of the method is that pigments are extracted from leaf tissues using 80% acetone, and their optical density is measured at wavelengths of 663 nm (chlorophyll "a") and 645 nm (chlorophyll "b") [10].

The measurements are calculated using specific formulas to determine the amounts of chlorophyll "a", chlorophyll "b", and total chlorophyll (mg/g leaf mass). To prevent pigment degradation due to light and temperature during the experiment, all procedures were conducted under dark and cold conditions.

This method plays an essential role in evaluating photosynthetic activity, physiological status, the influence of agronomic practices, and plant tolerance to stress factors. The obtained results serve as a reliable indicator for analyzing plant growth processes and determining the effect of different cultivars and environmental conditions.

Determination of chlorophyll "a", chlorophyll "b", and their total content in plant leaves is crucial for assessing photosynthetic activity (Figure 1).

Figure 1. The process of determining leaf pigments in *Ph. aureus* cultivars using spectrophotometry.

Equipment and reagents required for the experiment: Fresh, healthy leaf samples (0.2 - 0.5 g), 80% acetone solution, mortar and pestle (for leaf grinding), filter paper or centrifuge, pipette, test tubes, measuring cylinder, cuvettes (for spectrophotometer), spectrophotometer (or photo-colorimeter), distilled water, protective gloves and goggles.

For the experiment, 0.2 - 0.5 g of healthy, fully green leaves are collected. The leaves are ground in a mortar with 80% acetone (protected from light). The homogenized mixture is washed several times with 80% acetone, and extraction is continued until all pigments are isolated.

Filtration or Centrifugation. The obtained liquid is passed through filter paper or centrifuged at 3000 rpm for 10 minutes. The resulting clear filtrate is used for further analysis. All procedures are carried out in dark or semi-dark conditions to prevent chlorophyll degradation.

Spectrophotometric Measurement. An 80% acetone solution is used as a "blank", and the spectrophotometer is calibrated to zero. From the filtrate, 3 - 5 ml is poured into a cuvette. The optical density of the solution is measured at the following

wavelengths: 663 nm—for chlorophyll "a", 645 nm—for chlorophyll "b".

Measurements are repeated 2 - 3 times for each variant.

Calculations are performed using formulas developed by Arnon (1949) [10]:

$$\text{Chlorophyll "a" (mg/L)} = 12.7\ (A663) - 2.69\ (A645)$$

$$\text{Chlorophyll "b" (mg/L)} = 22.9\ (A645) - 4.68\ (A663)$$

$$\text{Total chlorophyll content (mg/L)} = 20.2\ (A645) + 8.02\ (A663)$$

If leaf mass is taken into account, the following formula is applied:

$$\text{Chl (mg/g)} = C \times V / 1000 \times W$$

where:

C—chlorophyll concentration (mg/L),

V—volume of extract (ml),

W—leaf mass (g).

The obtained values are calculated for each experimental variant. The amounts of chlorophyll "a", "b", and total chlorophyll are presented in tabular form. Differences among variants are analyzed statistically using ANOVA or a t-test. Results are illustrated using graphs or diagrams.

After sowing the *Ph. aureus* cultivars, the growth and developmental stages of the plant were monitored. Samples for pigment analysis were collected after the plants produced their first true leaves.

The developmental stages of the plant proceeded as follows: seed germination → cotyledon stage → formation of true leaves → branching → flowering → pod formation → seed maturation.

Four to six days after germination, the hypocotyl fully elongates, lifting the cotyledons above the soil surface. From between the cotyledons, the first true leaf appears in bud form. The first true leaf is simple, while subsequent leaves are compound (trifoliate). Each new leaf develops sequentially in a spiral arrangement. The young leaves are small, tender, and light green; over time, they thicken and become dark green.

When the plant produces 3 - 4 true leaves, the branching stage begins. The optimal temperature for true leaf formation is 25˚C - 30˚C. Under cold conditions, growth slows down. Leaf development is most active when soil moisture is at 60% - 70%. Adequate light accelerates chlorophyll synthesis in the leaves. In particular, phosphorus and nitrogen fertilizers significantly enhance leaf formation.

True leaves are considered the main photosynthetic organs of the plant. At this stage: chlorophyll synthesis intensifies in the leaf blades, the plant actively produces nutrients, rhizobacteria (nodule-forming bacteria) multiply rapidly in the roots and nitrogen fixation begins; the plant starts to accumulate vegetative biomass quickly.

In field experiments, the true leaf formation stage is taken as an important physiological indicator for determining the developmental phases of the plant.

This stage is usually observed 7 - 10 days after sowing, and differences among plants (variety, fertilizer rate, irrigation regime, climatic conditions) become most noticeable during this period (**Table 1**).

Table 1. Analysis of chlorophyll "a", chlorophyll "b" and carotenoid content of *Ph. aureus* plant.

Samples of varieties	470-chlorophyll-a			649-chlorophyll-b			664-carotenoid		
	$\bar{x}$ ± Sx	S	V%	$\bar{x}$ ± Sx	S	V%	$\bar{x}$ ± Sx	S	V%
NC-17376 China	1.068 ± 0.018	0.032	2.95	0.483 ± 0.054	0.094	19.37	0.852 ± 0.028	0.048	5.64
NC-17338 India	0.780 ± 0.022	0.039	4.97	0.278 ± 0.013	0.023	8.25	0.577 ± 0.024	0.042	7.32
NC-33641 Afghanistan	1.170 ± 0.121	0.209	17.86	0.415 ± 0.056	0.097	23.46	1.070 ± 0.265	0.458	42.82
NC-33068 Uzbekistan	1.183 ± 0.173	0.3	25.38	0.427 ± 0.058	0.101	23.52	0.908 ± 0.112	0.193	21.28
NC-32000 Vietnam	1.146 ± 0.081	0.14	12.24	0.533 ± 0.079	0.137	25.71	0.988 ± 0.060	0.103	10.46
NC-32061 Philippines	1.085 ± 0.026	0.046	4.21	0.508 ± 0.016	0.028	5.52	0.853 ± 0.033	0.058	6.75
NC-17385 Manchuria	1.139 ± 0.031	0.054	4.78	0.592 ± 0.055	0.095	15.97	0.923 ± 0.022	0.037	4.04
NC-17397 Vietnam	1.106 ± 0.008	0.014	1.23	0.578 ± 0.035	0.061	10.54	0.856 ± 0.022	0.037	4.36
NC-33647 Taiwan region	1.110 ± 0.007	0.012	1.04	0.545 ± 0.060	0.105	19.17	0.941 ± 0.025	0.044	4.63
NC-17393 China-2019	1.260 ± 0.192	0.332	26.34	0.447 ± 0.073	0.126	28.25	0.953 ± 0.138	0.239	25.07
Turon	1.225 ± 0.014	0.024	1.98	0.443 ± 0.002	0.003	0.73	0.923 ± 0.006	0.011	1.14
Durdona	1.087 ± 0.016	0.029	2.63	0.393 ± 0.023	0.04	10.11	0.823 ± 0.046	0.08	9.73

Ph. aureus is a thermophilic crop, and its seeds germinate within 5 - 7 days at a temperature of 12˚C - 15˚C. Seedlings are damaged at temperatures of −1˚C to −2˚C.

It is moisture-loving, and the seeds require an amount of water equal to their own weight to germinate. The plant especially needs more moisture during the budding stage.

It does not grow well in shaded areas and develops best in fertile meadow soils. The species is predominantly self-pollinating (Figure 2).

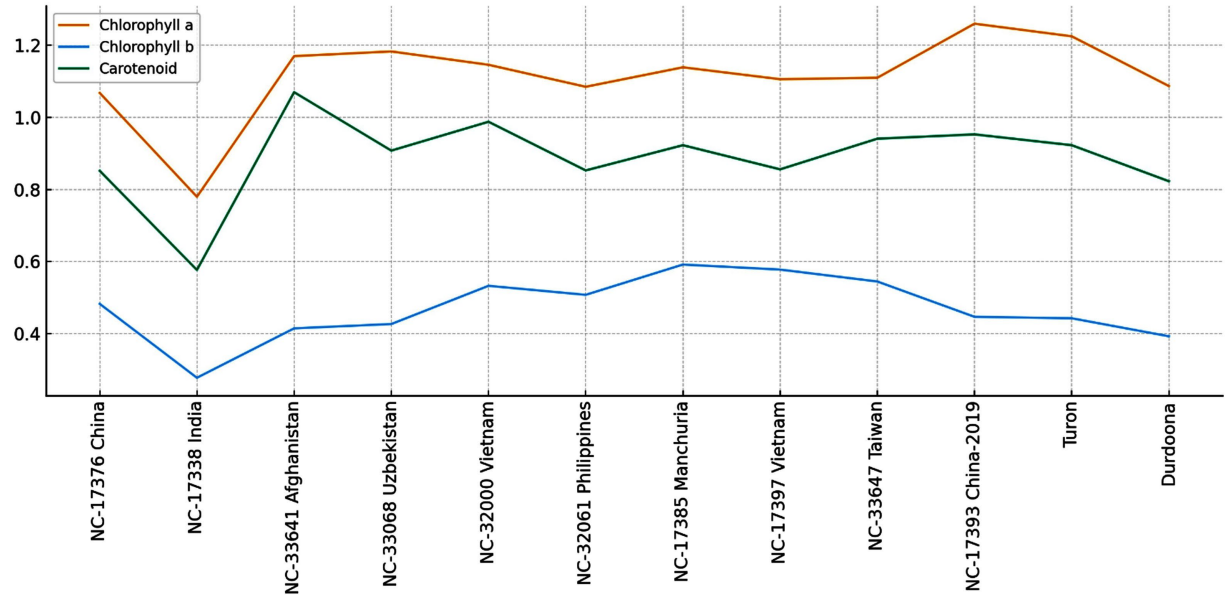

Figure 2. Analysis of leaf pigments in the true leaf stage of *Ph. aureus*.

Rhizobium bacteria in the root system fix atmospheric nitrogen, which contributes to the enrichment of the soil with organic matter [19].

The optimal growth temperature for the plant is 25˚C - 35˚C. Growth slows down at temperatures below 15˚C [10].

It prefers sandy or loamy, well-drained soils. A pH range of 6.0 - 7.0 is considered optimal. Moderate irrigation is required; excessive moisture leads to root rot [22].

In Uzbekistan, spring-sown crops mature within 85 - 95 days, while mung beans planted in late summer mature within 60 - 65 days. Harvesting is carried out when 75% - 80% of the pods are ripe [12].

Ph. aureus is a diploid plant with a chromosome number of 2n = 22. Its genome size is approximately 560 Mb.

In recent years, genome sequencing has enabled extensive studies on the genetic diversity and disease resistance of *Ph. aureus* [18].

The plant possesses C3-type photosynthesis, meaning that it is sensitive to high temperatures and drought stress [20].

It is rich in proteins (20% - 24%), carbohydrates (60% - 65%), fiber, and B-group vitamins [17].

Global cultivation and consumption of *Ph. aureus*. According to FAO (Food and Agriculture Organization of the United Nations), the global production of *Ph. aureus* has been steadily increasing. In recent years, more than 6 million tons of *Ph. aureus* have been produced worldwide. Over 90% of the total production comes from Asian countries.

Ph. aureus is most widely consumed in India, which accounts for more than 60% of the global cultivation area. China, Myanmar, North Korea, Thailand, Indonesia, Pakistan, and Uzbekistan follow as leading producers [12].

The plant is moderately tolerant to drought but sensitive to saline soils [18].

Ph. aureus plays an important role in improving soil fertility due to nitrogen fixation, which enriches the soil with organic compounds [19].

It is used as food (beans, sprouts, flour), animal feed, and green manure [22].

Connecting High Carotenoid Content to Stress Tolerance in *Ph. aureus.* The results of this study revealed that certain accessions of *Ph. aureus*, particularly those from Afghanistan and Uzbekistan, exhibited notably high carotenoid levels. Carotenoids play a critical role in photoprotection by quenching excess light energy and scavenging reactive oxygen species generated under high light or drought stress. This protective function reduces photo-oxidative damage to the photosynthetic apparatus, ensuring more stable photosynthetic activity under adverse environmental conditions.

The elevated carotenoid content observed in these accessions likely contributes to their enhanced tolerance to arid and semi-arid environments. By mitigating light-induced stress, carotenoids support overall plant resilience, growth, and productivity, aligning with the known ability of *Ph. aureus* to thrive in regions with limited water availability. Therefore, the findings provide a mechanistic link between

pigment composition and ecological adaptability, strengthening the understanding of how biochemical traits contribute to stress tolerance in this species.

4. Conclusions

The comparative analysis of chlorophyll "a", chlorophyll "b", and carotenoid contents in the examined *Ph. aureus* varieties revealed substantial biochemical variability among accessions from different geographical regions. Chlorophyll "a" and "b" concentrations, along with carotenoid levels, were generally higher in accessions originating from regions with strong solar radiation, such as Afghanistan, Uzbekistan, and China, indicating enhanced photosynthetic potential and photoprotective capacity.

These findings have important practical implications for breeding programs. Breeders can utilize this information to select high-pigment genotypes—particularly those from Afghanistan and Uzbekistan—with elevated carotenoid and chlorophyll contents to develop new varieties that are more resilient to environmental stress, including high light intensity and drought conditions. Such targeted selection can enhance both the stress tolerance and productivity of *Ph. aureus*, while also providing a biochemical basis for improving cultivar performance under diverse agroecological conditions.

Overall, the study underscores the value of pigment profiling as a tool for identifying physiologically robust genotypes and guiding the development of improved, stress-adapted cultivars.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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